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An Electrochemical Study on Chlorhexidine Gluconate Addition to Normal Saline for Oral Implant Applications

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Chlorhexidine gluconate (CHG) is an effective bactericidal agent commonly used in periodontics and oral and maxillofacial procedures. Upon the application of CHG in these procedures, an implant or prosthesis can come in contact with CHG which might alter the electrochemistry at the metal-body fluid interface. The effect of 0.01% CHG addition to normal saline (NS) on the corrosion behavior of Ti1 implant alloy has been investigated using various electrochemical techniques to simulate the use of CHG in the oral environment. Electrochemical testing was performed at 298 K under naturally aerated conditions using techniques such as open circuit potential (OCP), electrochemical impedance spectroscopy (EIS), potentiodynamic and potentiostatic measurements. On addition of CHG to normal saline, the corrosion rate of Ti1 alloy increases, more so with increasing exposure time. EIS analysis reveals the presence of two time constants in the presence of CHG in contrast to a one time constant in pure normal saline. It is recommended to use CHG in the presence of a corrosion inhibitor for its safe use in various oral procedures on patients with titanium implants/prostheses.

Keywords: chlorhexidine gluconate, corrosion, dental implant, titanium alloy

1. INTRODUCTION

Amongst the commercially available dental implant materials for several stomatognathic, maxillofacial and craniofacial applications, titanium alloys are the most commonly used [1-4]. The biocompatibility of the metallic implant structure has been attributed to the thermodynamic, mechanical and electrochemical stability [3-4] of the various oxides and sub-oxides formed over its surface, primarily the n-type semiconducting TiO_2 oxide layer [5-6].

Osteo-integration for any implantation inside the human body chiefly depends upon the interactions between the cells and implant surface and is governed by a number of different physical and chemical processes. Surface topography [7-9], surface chemistry [9-14], metal-biofluid interactions in capsular space and metal-tissue and tissue-biofluid interactions [15] appear to play a substantial role in the final prognosis of the surgical procedure. Thus, operative and post-operative procedures can contribute to a critical change in surface characteristics, hampering the success for effective osteo-integration.

Chlorhexidine gluconate [16] is a cationic bis-biguanide molecule, designated as 1,1'-hexamethylenebis [5-(p-chlorophenyl)biguanide] di-D-gluconate (Figure 1).

Figure 1. Chlorhexidine gluconate

It is a broad spectrum antimicrobial agent, effective against majority of gram-positive, gram-negative bacteria, yeast and algae [17-20]. Studies have shown reductions in the numbers of the putative periodontopathic organisms like porphyromonas gingivalis, prevotella intermedia, bacteriodes forsythus, and campylobacter species following CHG periodontal and surgical therapies [17-20]. CHG is used in various concentrations varying between 0.01% to 2% depending upon treatment protocol and prophylactic regimen. 0.01% CHG is usually recommended for daily mouth rinse regimen to prevent substantivity and sensitivity and hence has been chosen for the study [18].

To envisage a favorable prognosis after the oral-prophylactic or surgical procedures, it is of paramount importance to investigate the electrochemistry at the implant-CHG interface. The aim of the present investigation is to hence investigate the electrochemical response of Ti1 alloy in the presence of 0.01% CHG in normal saline solution.

2. EXPERIMENTAL

2.1. Materials Preparation

Commercially pure titanium alloy grade Ti1 (0.1%C, 0.2%Fe, 0.015%H, 0.03%N, 0.18%O & 99.47% Ti) was used for the present investigation. Available cuboidal rod was cut, soldered to a copper wire and mounted in an epoxy resin with exposed cross section area of 0.855 cm^2 for use as a working electrode. The working electrode was finished with various grades of silicon carbide papers upto 2400 and ultrasonically cleaned with de-ionized water and acetone. Normal saline solution (5.26 gL^{-1} sodium chloride, 2.22 gL^{-1} anhydrous sodium acetate, 5.02 gL^{-1} sodium gluconate, 0.37 gL^{-1} potassium chloride and 0.3 gL^{-1} magnesium chloride hexahydrate) (NS) of pH 6.6 was used as a simulated body fluid and a control solution. Chlorhexidine gluconate (4% solution (Hibiclens)) was used to prepare 0.01% CHG solution in normal saline solution.

2.2. Experimental Set-up & Measurements

A three-electrode cell assembly consisting of Ti1 alloy as the working electrode, platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode was used to perform electrochemical measurements in pure normal saline and in 0.01% CHG solution. The experiments were conducted at 298 K and under naturally aerated conditions. The DC electrochemical measurements were conducted using a PAR Potentiostat 273A and for AC measurements, a PAR 1255 FRA was used in addition.

The electrochemical measurements were performed in the following sequence:

Open Circuit Potential (OCP): OCP values of the alloy were monitored from immersion to various time intervals such as 6, 24, 48 and 168 hours.

Electrochemical Impedance Spectroscopy (EIS): Non destructive impedance measurements were conducted at similar time intervals as above. The frequency sweep was applied from 10^5 to 10^{-2} Hz at the OCP with the superimposition of 10 mV AC amplitude.

Potentiodynamic Polarization: Potentiodynamic polarization tests were performed at 168 hours of immersion. The polarization sweep was applied from -500 mV vs. OCP upto 1 V vs. SCE using a scan rate of 1 mV/s.

Potentiostatic Polarization: Potentiostatic polarization sweep was applied in continuation to the potentiodynamic polarization at 1 V upto 3600 seconds.

3. RESULTS

The evolution of OCP with time for Ti1 in normal saline and in 0.01% CHG has been shown in Fig. 2. The OCP values for 0.01% CHG are observed to be more positive to those for normal saline.

The EIS plots for Ti1 in normal saline were found to follow a one-time constant circuit model as shown in Fig. 3a and the plots in the presence of CHG followed a two-time constant impedance response as shown in Fig. 3b.

The one-time constant circuit model represents a compact oxide film formed on the titanium surface [21], where R_s is the solution resistance, R_b is the compact barrier layer resistance and CPE_b is the constant phase element for the capacitance of the barrier layer. To deal with capacitance dispersion, CPE has been used in place of capacitance due to the non-ideal capacitive response of the oxide film [22-23].

The two-time constant circuit model shown in Fig. 3b represents an oxide film with pores [22]. The part of the oxide film that is still compact acts as a barrier layer and the outer part of the oxide which has developed pores constitutes the porous layer. The CPE_p and R_p represent the capacitance and the resistance of the porous layer. Instead of CPE, a pure capacitor has been used to represent the capacitance of the inner barrier layer as it was found to give a better fit compared to a CPE.

Fig. 4 presents the impedance plots for Ti1 in normal saline and in 0.01% CHG at one hour and 168 hours of immersion. The impedance response shows a low corrosion resistance in the presence of 0.01% CHG than in pure normal saline at both immersion hours. The impedance parameters derived from circuit modeling for Ti1 in normal saline and in the presence of 0.01% CHG have been listed in Tables 1 and 2.

Fig. 5 and Fig. 6 compare the potentiodynamic and potentiostatic curves for Ti1 in normal saline and in 0.01% CHG at 168 hours of immersion. Various polarization parameters deduced from these curves have been listed in Table 3. The corrosion current densities were obtained from the polarization curves by extrapolation of the cathodic branch of the polarization curves to the corrosion potential.

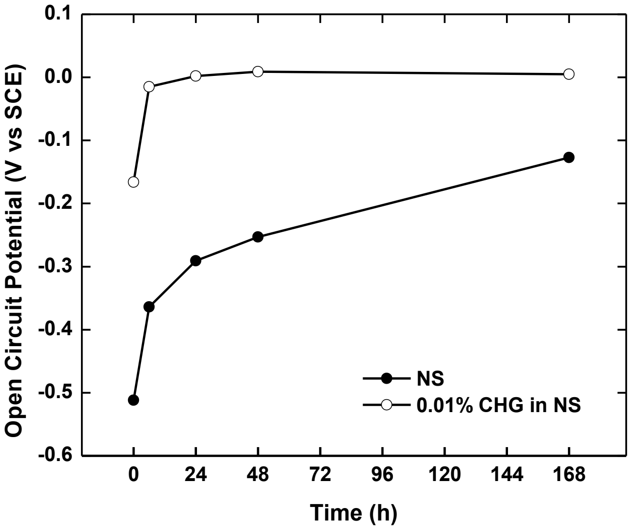
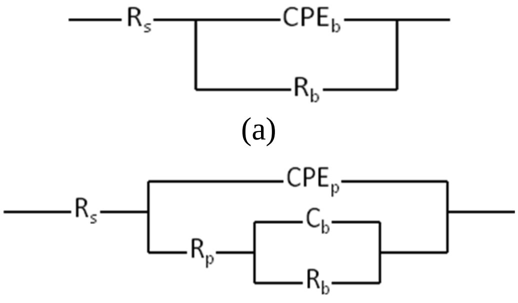


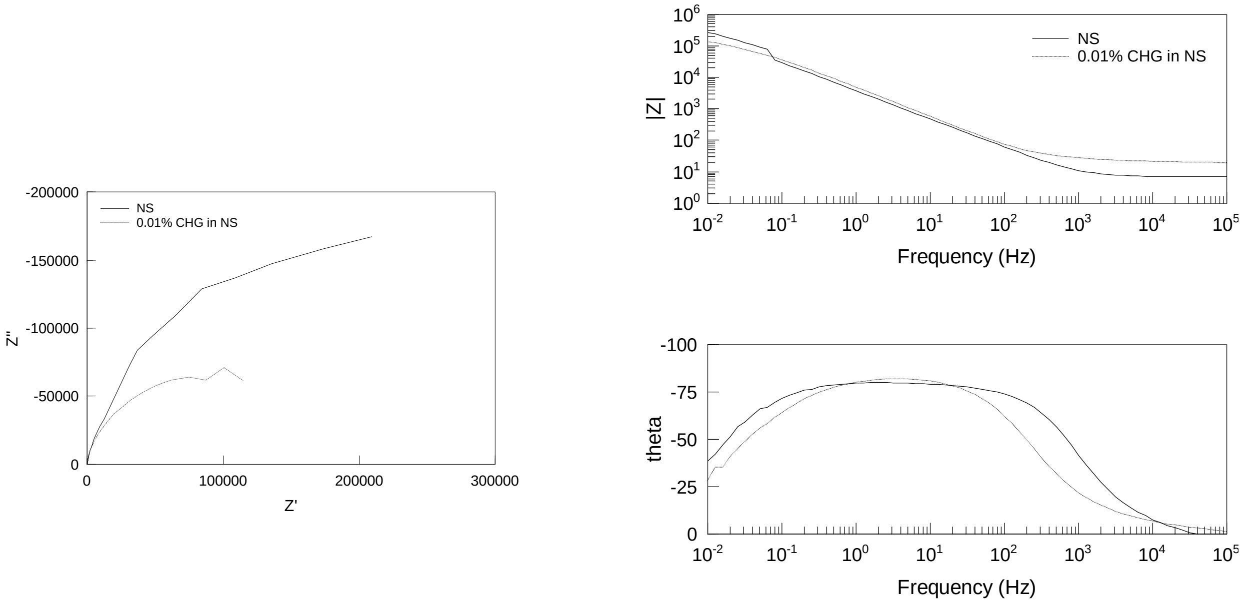
Figure 2. Open circuit potential as a function of time for Ti1 in normal saline and 0.01% CHG



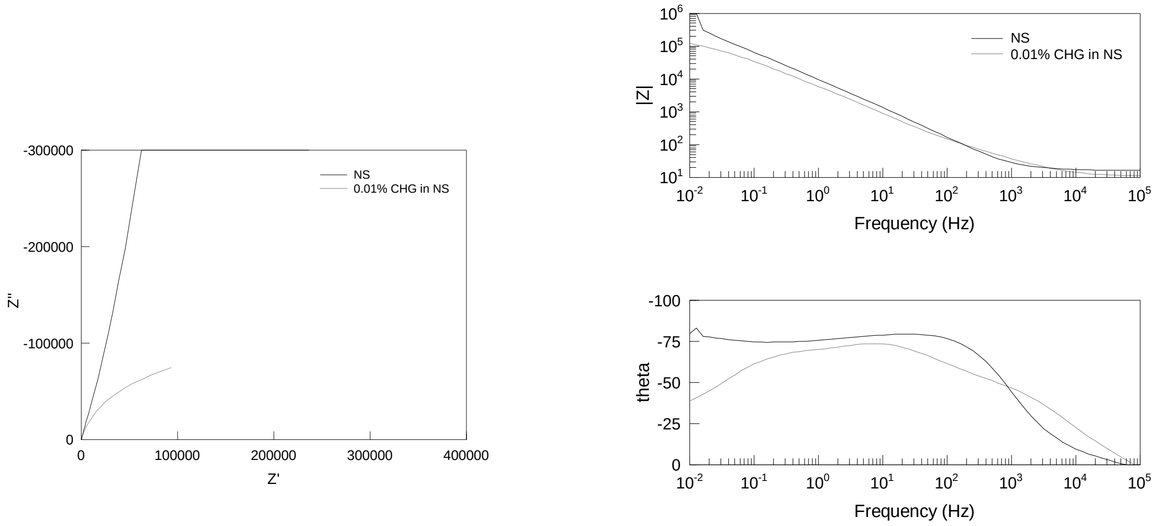
(a)

(b)

Figure 3. Circuit models used to fit EIS results for Ti1 in (a) normal saline (b) 0.01% CHG



(a) At 1 hour of immersion



(b) At 168 hours of immersion

Figure 4. Impedance plots (Nyquist & Bode) for Ti1 in normal saline and 0.01% CHG at various immersion hours

Table 1. Impedance parameters for Ti1 in normal saline at various immersion hours

Immersion time (h)	R_s (Ω cm^2)	R_b ($x10^6$) (Ω cm^2)	CPE_b ($x10^{-5}$) ($Scm^{-2}(s\ rad^{-1})^n$)	n_b
1	7.06	0.33	4.11	0.93
6	7.42	1.13	2.96	0.91
24	9.18	3.29	2.80	0.89
48	9.09	4.57	2.74	0.88
168	16.16	3.55	2.20	0.87

Table 2. Impedance parameters for Ti1 in 0.01% CHG at various immersion hours

Immersion time (h)	R_s (Ω cm^2)	R_b (x10^5) (Ω cm^2)	C_b (x10^{-6}) (S cm^{-2} s rad^{-1})	R_p (Ω cm^2)	CPE_p (x10^{-5}) (Scm^{-2}(s rad^{-1})^n)	n_p
1	19.88	1.4	11.3	23.12	2.91	0.86
6	7.47	2.05	4.74	82.57	2.68	0.86
24	11.11	1.84	5.16	95.46	2.55	0.82
48	14.11	1.71	5.33	126.6	2.93	0.79
168	10.79	1.9	3.92	240.4	3.69	0.76

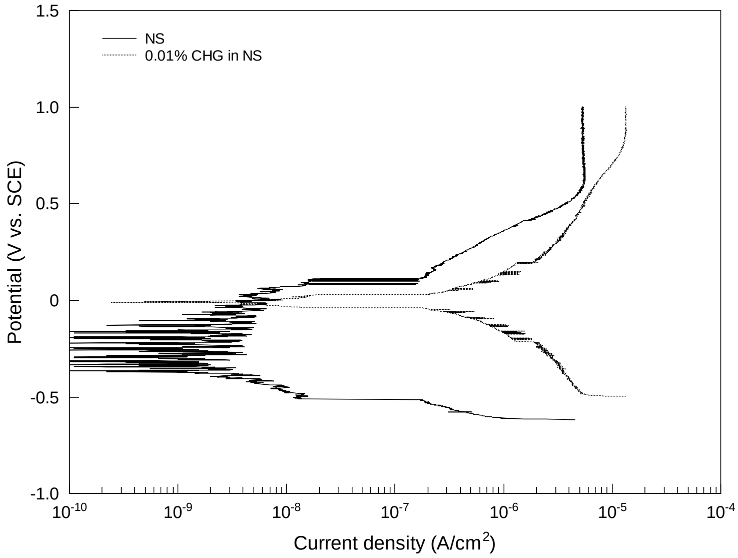


Figure 5. Potentiodynamic curves for Ti1 at 168 hours of immersion

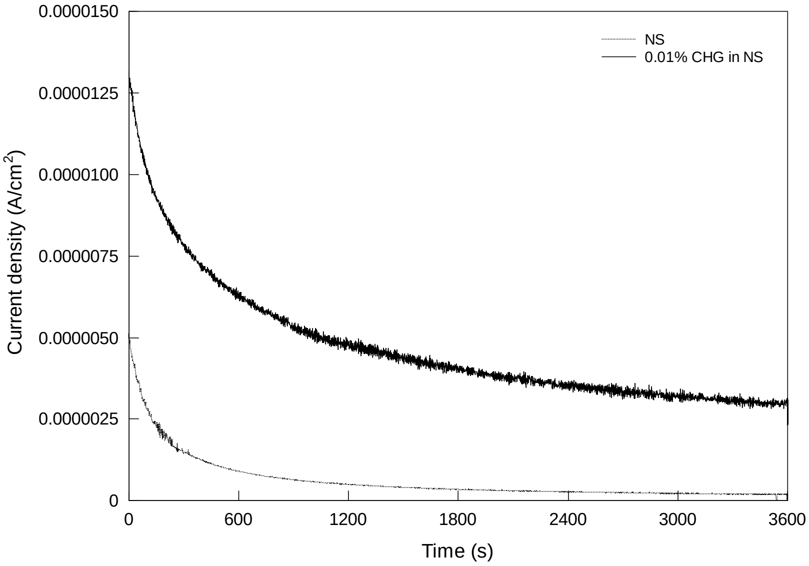


Figure 6. Potentiostatic curves for Ti1 at 168 hours of immersion

Table 3. Polarization parameters for Ti1 in normal saline and 0.01% CHG at 168 hours of immersion

Solution	i_{corr} (x 10^{-7}) (A/cm^2)	E_{corr} (V vs. SCE)	Steady state current density (x 10^{-7}) (A/cm^2)
NS	0.103	-0.367	1.79
0.01% CHG in NS	9.40	-0.009	30.0

4. DISCUSSION

The more positive OCP values for Ti1 in the presence of CHG compared to pure normal saline can be explained from the analysis of the polarization curves, as discussed in the later part of this section.

The OCP values are also observed to show an increase with increasing immersion time and the increase is more prominent in case of normal saline. The increase in OCP values with time for titanium alloys in simulated body solutions is generally due to the formation of stable passive oxide layers [24].

In Fig. 4a, the Nyquist plot in the presence of CHG at 1 hour of immersion shows lower impedance values (smaller semicircle diameter) as compared to pure normal saline. At 168 hours of immersion (Fig. 4b), the difference in the impedance values for normal saline and CHG increases compared to 1 hour, which is evident from both the Bode (phase angle and impedance) and Nyquist plots in figures 4a and 4b.

The phase angle Bode curves for normal saline at 1 hour and at 168 hours appear to be quite different. The phase angles in Fig. 4a show a decrease in the low frequency region, while the phase angles in Fig. 4b display a near capacitive response throughout the intermediate and the low frequency regions with constant phase angles. This is suggestive of the highly compact nature of the oxide film grown on Ti1 in normal saline in an immersion time of 168 hours as compared to 1 hour. In Table 1, it is observed that in case of normal saline, the barrier layer resistance increases with increase in immersion time and the capacitance of the oxide correspondingly decreases. The barrier layer resistance shows a tenfold increase from 1 to 168 hours of immersion which corresponds to the phase angle changes observed in Fig. 4a and Fig. 4b. One time constant circuit model corresponding to a compact oxide has been used to simulate the behavior of Ti1 in normal saline.

However, in case of CHG, at both immersion hours, the shapes of the phase angle curves are similar. The only difference in the phase angle curves is the noticeable separation of the two time constants at 168 hours which is not evident at 1 hour when the two time constants are superimposed. In the presence of CHG, in Table 2, impedance parameters following a two time constant model of an inner barrier and outer porous layer have been listed. The R_b values are an order of magnitude lower than the R_b values in case of normal saline. It appears that upon CHG addition to normal saline, the molecules interferes with the oxide growth in solution and increase the charge transfer kinetics at the oxide/solution interface. This process makes the outer part of the oxide porous, showing up as another time constant in the impedance plots. The outer porous layer can be together comprised of the oxide and adsorbed CHG and other ions from the solution. The increase in the adsorption of CHG molecules and the formation of corrosion products produces an increase in R_p with immersion time.

In case of CHG, the capacitance of the inner barrier layer has been represented by a pure capacitor instead of a CPE, which implies that the inner barrier layer in this case is more homogeneous compared to that in normal saline. This could be due to the formation of a separate outer porous layer with greater roughness and heterogeneity, which has originated from the outer portion of the barrier layer, thus leaving behind a smoother surface. The capacitance values of the barrier layer are an order of magnitude lower than those for normal saline. This could be due to the decrease in the effective area of the barrier layer oxide surface due to surface smoothening. The inner barrier layer has a much higher resistance as compared to the outer porous layer and is thus responsible for the corrosion resistance of the alloy.

The capacitance values of the barrier and the porous layers shown in tables 1 and 2 already have contributions from the Helmholtz double layer capacitance which is considered as a capacitance in series with the barrier/porous layer capacities. A constant value of 20 $\mu F\ cm^{-2}$ is usually assumed to account for the capacitance of the double layer [25-26].

The positive OCP values of Ti1 observed in Fig. 2 in the presence of CHG are hence as a result of the changes observed in the anodic and cathodic branches of the potentiodynamic curves shown in Fig. 5. Current densities corresponding to both the branches appear to have increased upon CHG addition, leading to an increase in the corrosion current density of Ti1. A greater increase in cathodic current densities has caused the shift in OCP values in the positive direction.

Oztan et al. [27] have reported severe corrosion of stainless steels files in the presence of 0.2% chlorhexidine gluconate. In the present case, even 0.01% CHG appears to cause an increase in the corrosion rate of Ti1 alloy.

The presence of a compact oxide surface film on titanium results in a multilayered interface consisting of metal/compact surface film/double layer/solution interface [28].

At the metal/oxide interface, oxidation of titanium takes place:

$$Ti \rightarrow Ti^{2+} + 2e^- \qquad (1)$$

At the oxide/solution interface, the electrons released are taken up by the corresponding cathodic reaction at near-neutral pH:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \qquad (2)$$

and dissociation of water takes place according to:

$$2H_2O \rightarrow 2O^{2-} + 4H^+ \qquad (3)$$

The overall reaction at both interfaces is the formation of TiO_2:

$$Ti^{2+} + 2O^{2-} \rightarrow TiO_2 + 2e^- \qquad (4)$$

TiO_2 oxide layer further undergoes the following electrochemical oxidation reactions in the simulated body fluid:

$$Ti + 3OH^- \rightarrow Ti(OH)_3 + 3e^- \quad (5)$$

$$Ti(OH)_3 \rightarrow TiO_2.H_2O + H^+ + e^- \quad (6)$$

Rosca et al. [29] have proposed a passivation mechanism in chloride containing solutions for titanium,

$$Ti + 4Cl^- \rightarrow [TiCl_4]^- \rightarrow TiCl_4 + 4e^- \quad (7)$$

$$TiCl_4 + 2H_2O \rightarrow TiO_2 + 4Cl^- + 4H^+ \quad (8)$$

According to the authors, the chemisorbed chloride complex anion, $[TiCl_4]^-$, is transformed to $TiCl_4$, which rapidly hydrolyses forming the TiO_2 passive film.

Titanium is also known to undergo complex formation with gluconate [30-31]. The complexation reaction can promote increasing dissolution of titanium oxide producing an increase in anodic current densities in the presence of CHG.

Chlorhexidine is a cationic bis-biguanide, consisting of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a hexamethylene chain as shown in Fig. 1. Chlorhexidine is a hydrophobic and lipophilic molecule. Chlorhexidine gluconate salt is a water soluble form of chlorhexidine and at physiological pH, readily dissociates and releases the positively charged chlorhexidine component [32]. It has been further reported that at the physiological pH, chlorhexidine is protonated and assumes a high positive charge. Its cationic properties account for its strong affinity for negatively charged groups on oral surfaces, which form a basis for its antibacterial efficacy [33].

The positively charged chlorhexidine molecule has an affinity for negatively charged groups and the hydroxyl ions formed in the cathodic reaction 2 can be electrostatically attracted towards the positively charged chlorhexidine molecules, which can accelerate the cathodic reaction in the forward direction. This can also hinder the rates of reactions 5 and 6, creating a barrier towards the growth of oxide in solution by favoring the backward reaction. Moreover, chlorhexidine can also interact with the chloride ions in solution which can again impede the formation of oxide by reactions 7 and 8.

According to the potential-pH diagram given by Black, the potential of a metallic biomaterial can vary up to 1.2 V under physiological conditions [34]. Potentiostatic curves (Fig. 6) were thus generated for Ti1 at 168 hours of immersion at an impressed potential of 1V in the passive region. The curves exhibit a rapid decrease in current density as a function of time followed by a slow decay to attain a steady state current value. This is explained by the decrease in active area due to the growth of the oxide film in the passive range [35-36]. The steady state current values are lower for normal saline compared to CHG as is also observed in Table 3, which indicates that in the passive range also, the anodic oxide formed over Ti1 alloy in the presence of CHG offers less resistance to dissolution compared to pure normal saline.

5. CONCLUSIONS

Chlorhexidine Gluconate is an effective bactericidal agent universally used in routine periodontics (oral prophylaxis) and oral and maxillofacial procedures. The addition of CHG to normal saline increases the corrosion of the titanium implant both at immersion and increasing exposure hours, with increased exposure showing more severity of attack. This corrosive attack may bring about local or systemic effects in the host, thereby complicating the treatment procedure. It is therefore highly recommended to perform a proper selection of the bactericidal agent in consideration to its corrosive activity for use in patients with titanium implants or prostheses. If the use of CHG is inevitable in some oral therapy, it should be applied in the presence of a suitable corrosion inhibitor.

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